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L1: Entry 1 of 1 File: USPT Nov 6, 2001

US-PAT-NO: 6312689
DOCUMENT-IDENTIFIER: US 6312689 B1

TITLE: Anti-CCR2 antibodies and methods of use therefor

DATE-ISSUED: November 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
LaRosa; Gregory J.	West Roxbury	MA		

US-CL-CURRENT: 424/130.1; 424/141.1, 424/143.1, 424/159.1, 530/388.22, 530/388.23, 530/389.2

CLAIMS:

What is claimed is:

1. An antibody or antigen-binding fragment thereof which binds to a mammalian CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a chemokine to said receptor and inhibits one or more functions associated with binding of the chemokine to said receptor, and wherein said antibody or antigen-binding fragment thereof binds the amino-terminal domain of said receptor.

2. An antibody or antigen-binding fragment thereof according to claim 1 wherein said antibody or antigen-binding fragment binds a portion of the amino-terminal domain which is from about amino acid 1 to about amino acid 30 of said receptor.

3. An antibody or antigen-binding fragment thereof according to claim 1 wherein the antibody is selected from the group consisting of:

a) monoclonal antibody 1D9;

b) an antibody having the epitopic specificity of 1D9;

c) monoclonal antibody 8G2;

d) an antibody having the epitopic specificity of 8G2; and

e) antigen-binding fragments of any one of (a) through (d) which bind to mammalian CC-chemokine receptor 2 or a portion thereof.

4. An antibody or antigen-binding fragment thereof according to claim 1 wherein the chemokine is selected from the group consisting of MCP-1, MCP-2, MCP-3, MCP-4 and combinations thereof.

5. A composition comprising an antibody or antigen-binding fragment thereof which binds to a mammalian CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a chemokine to said receptor and inhibits one or more functions associated with binding of the chemokine to said receptor, and wherein said antibody or antigen-binding fragment thereof binds the amino-terminal domain of said receptor, and an optional physiologically acceptable vehicle.

6. A method of treating a CC-chemokine receptor 2-mediated disorder in a patient, comprising administering to the patient an effective amount of an antibody or antigen-binding fragment thereof which binds to mammalian CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a chemokine to said receptor and inhibits one or more functions associated with binding of the chemokine to said receptor, and wherein said antibody or antigen-binding fragment thereof binds the amino-terminal domain of said receptor.

7. A method according to claim 6, wherein said CC-chemokine receptor 2-mediated disorder is an autoimmune disorder.

8. A method according to claim 7, wherein the autoimmune disorder is selected from the group consisting of multiple sclerosis and rheumatoid arthritis.

9. A method according to claim 8, wherein the autoimmune disorder is multiple sclerosis.

10. A method according to claim 6, wherein the CC-chemokine receptor 2-mediated disorder is selected from the group consisting of atherogensis and atherosclerosis.

11. An antibody or antigen-binding fragment according to claim 1, wherein said antibody or fragment is a monoclonal antibody or fragment thereof.

12. An antibody or antigen-binding fragment according to claim 1, wherein said antibody or fragment is a human antibody or fragment thereof.

13. An antibody or antigen-binding fragment according to claim 1, wherein said antigen-binding fragment is selected from the group consisting of an Fv fragment, an Fab fragment, an Fab' fragment and an F(ab').sub.2 fragment.

14. An antibody or antigen-binding fragment thereof which binds to a mammalian CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a chemokine to said receptor and inhibits one or more functions associated with binding of the chemokine to said receptor, and wherein said antibody or antigen-binding fragment thereof can compete with monoclonal antibody 1D9 for binding to said receptor.

15. An antibody or antigen-binding fragment thereof according to claim 14, wherein said mammalian CC-chemokine receptor 2 is a human CC-chemokine receptor 2.

16. An antibody or antigen-binding fragment thereof according to claim 14, wherein the chemokine is selected from the group consisting of MCP-1, MCP-2, MCP-3, MCP-4 and combinations thereof.

17. A composition comprising an antibody or antigen-binding fragment thereof which binds to a mammalian CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a chemokine to said receptor and inhibits one or more functions associated with binding of the chemokine to said receptor, and wherein said antibody or antigen-binding fragment thereof can compete with monoclonal antibody 1D9 for binding to said receptor, and an optional physiologically acceptable vehicle.

18. A pharmaceutical composition comprising an antibody or antigen-binding fragment thereof which binds to a mammalian CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a chemokine to said receptor and inhibits one or more functions associated with binding of the chemokine to said receptor, and wherein said antibody or antigen-binding fragment thereof can compete with monoclonal antibody 1 D9 for binding to said receptor, and a physiologically acceptable vehicle.

19. An antibody or antigen-binding fragment according to claim 14, wherein said antibody or fragment is a monoclonal antibody or fragment thereof.

20. An antibody or antigen-binding fragment according to claim 14, wherein said antibody or fragment is a human antibody or fragment thereof.

21. An antibody or antigen-binding fragment according to claim 14, wherein said antigen-binding fragment is selected from the group consisting of an Fv fragment, an Fab fragment, an Fab' fragment and an F(ab').sub.2 fragment.

22. A composition comprising an antibody or antigen-binding fragment thereof which binds to a mammalian CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a chemokine to said receptor and inhibits one or more functions associated with binding of the chemokine to said receptor, and wherein said antibody or antigen-binding fragment thereof can compete with monoclonal antibody 8G2 for binding to said receptor, and an optional physiologically acceptable vehicle.

23. A pharmaceutical composition comprising an antibody or antigen-binding fragment thereof which binds to a mammalian CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a chemokine to said receptor and inhibits one or more functions associated with binding of the chemokine to said receptor, and wherein said antibody or antigen-binding fragment thereof can compete with monoclonal antibody 8G2 for binding to said receptor, and a physiologically acceptable vehicle.

24. An antibody or antigen-binding fragment thereof which binds to a mammalian CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a chemokine to said receptor and inhibits one or more functions associated with binding of the chemokine to said receptor, and wherein said antibody or antigen-binding fragment thereof can compete with monoclonal antibody 8G2 for binding to said receptor.

25. An antibody or antigen-binding fragment thereof according to claim 24, wherein said mammalian CC-chemokine receptor 2 is a human CC-chemokille receptor 2.

26. An antibody or antigen-binding fragment thereof according to claim 24, wherein the chemokine is selected from the group consisting of MCP-1, MCP-2, MCP-3, MCP-4 and combinations thereof.

27. An antibody or antigen-binding fragment according to claim 24, wherein said antibody or fragment is a monoclonal antibody or fragment thereof.

28. An antibody or antigen-binding fragment according to claim 24, wherein said antibody or fragment is a human antibody or fragment thereof.

29. An antibody or antigen-binding fragment according to claim 24, wherein said antigen-binding fragment is selected from the group consisting of an Fv fragment, an Fab fragment, an Fab' fragment and an F(ab').sub.2 fragment.

30. An antibody or antigen-binding fragment thereof which binds to a mammalian CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a ligand to the receptor and inhibits

one or more functions associated with binding of the ligand to the receptor at a concentration of less than about 10 .mu.g/ml.

31. An antibody or antigen-binding fragment thereof which binds to a mammalian CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a ligand to the receptor and inhibits one or more functions associated with binding of the ligand to the receptor at a concentration of less than about 0.1 .mu.g/ml.

32. An antibody or antigen-binding fragment thereof which binds to a human CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a chemokine to said receptor and inhibits one or more functions associated with binding of the chemokine to said receptor, and wherein said antibody or antigen-binding fragment thereof binds the amino-terminal domain of said receptor.

33. An antibody or antigen-binding fragment according to claim 32, wherein said antibody or fragment is a monoclonal antibody or fragment thereof.

34. An antibody or antigen-binding fragment according to claim 32, wherein said antibody or fragment is a human antibody or fragment thereof.

35. An antibody or antigen-binding fragment thereof according to claim 32, wherein the chemokine is selected from the group consisting of MCP-1, MCP-2, MCP-3, MCP-4 and combinations thereof.

36. A pharmaceutical composition comprising an antibody or antigen-binding fragment thereof which binds to a mammalian CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a chemokine to said receptor and inhibits one or more functions associated with binding of the chemokine to said receptor, and wherein said antibody or antigen-binding fragment thereof binds the amino-terminal domain of said receptor, and a physiologically acceptable vehicle.

37. A composition comprising an antibody or antigen-binding fragment thereof which binds to a human CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a chemokine to said receptor and inhibits one or more functions associated with binding of the chemokine to said receptor, and wherein said antibody or antigen-binding fragment thereof binds to the amino-terminal domain of said receptor, and an optional physiologically acceptable vehicle.

38. A pharmaceutical composition comprising an antibody or antigen-binding fragment thereof which binds to a human CC-chemokine receptor 2, wherein said antibody or antigen-binding fragment thereof inhibits binding of a chemokine to said receptor and inhibits one or more functions associated with binding of the chemokine to said receptor, and wherein said antibody or antigen-binding fragment thereof binds to the amino-terminal domain of said receptor, and a physiologically acceptable vehicle.

39. A method according to claim 6, wherein said CC-chemokine receptor 2-mediated disorder is asthma.

40. A method according to claim 8, wherein the autoimmune disorder is multiple sclerosis.

41. A method according to claim 8, wherein the autoimmune disorder is rheumatoid arthritis.

42. A method according to claim 10, wherein the CC-chemokine receptor 2-mediated disorder is atherogenesis.

43. A method according to claim 10, wherein the CC-chemokine receptor

2-mediated disorder is atherosclerosis.

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Search Results - Record(s) 1 through 8 of 8 returned.

☐ 1. Document ID: US 6448021 B1

L2: Entry 1 of 8 File: USPT Sep 10, 2002

US-PAT-NO: 6448021
DOCUMENT-IDENTIFIER: US 6448021 B1

TITLE: Method of inhibiting cell function associated with CCR2 by anti-CCR2 amino-terminal domain antibodies

DATE-ISSUED: September 10, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
LaRosa; Gregory J.	West Roxbury	MA		

US-CL-CURRENT: 435/7.1; 424/141.1, 435/345, 435/5, 435/7.93, 435/7.94

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☐ 2. Document ID: US 6406865 B2

L2: Entry 2 of 8 File: USPT Jun 18, 2002

US-PAT-NO: 6406865
DOCUMENT-IDENTIFIER: US 6406865 B2

TITLE: Method of inhibiting interaction of cells bearing CCR2 by Anti-CCR2 amino-terminal domain antibodies

DATE-ISSUED: June 18, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
LaRosa; Gregory J.	West Roxbury	MA		

US-CL-CURRENT: 435/7.1; 424/141.1, 435/345, 435/5, 435/7.93, 435/7.94

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☐ 3. Document ID: US 6406694 B1

L2: Entry 3 of 8 File: USPT Jun 18, 2002

US-PAT-NO: 6406694
DOCUMENT-IDENTIFIER: US 6406694 B1

TITLE: Anti-CCR2 antibodies

DATE-ISSUED: June 18, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
LaRosa; Gregory J.	West Roxbury	MA		

US-CL-CURRENT: 424/130.1; 424/134.1, 424/141.1, 424/143.1, 424/85.1, 530/388.22, 530/388.23, 530/389.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

☐ 4. Document ID: US 6395497 B1

L2: Entry 4 of 8 File: USPT May 28, 2002

US-PAT-NO: 6395497
DOCUMENT-IDENTIFIER: US 6395497 B1

TITLE: Method of inhibiting leukocyte trafficking by anti-CCR2 amino-terminal domain antibodies

DATE-ISSUED: May 28, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
LaRosa; Gregory J.	West Roxbury	MA		

US-CL-CURRENT: 435/7.1; 424/141.1, 435/345, 435/5, 435/7.93, 435/7.94

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

☐ 5. Document ID: US 6352832 B1

L2: Entry 5 of 8 File: USPT Mar 5, 2002

US-PAT-NO: 6352832

DOCUMENT-IDENTIFIER: US 6352832 B1

TITLE: Anti-CCR2 antibodies and methods of use therefor

DATE-ISSUED: March 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
LaRosa; Gregory J.	West Roxbury	MA		
Horvath; Christopher	Taunton	MA		
Newman; Walter	Boston	MA		

US-CL-CURRENT: 435/7.1; 435/343, 435/343.2, 435/345, 435/5, 436/548

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 6. Document ID: US 6312689 B1

L2: Entry 6 of 8 File: USPT Nov 6, 2001

US-PAT-NO: 6312689
DOCUMENT-IDENTIFIER: US 6312689 B1

TITLE: Anti-CCR2 antibodies and methods of use therefor

DATE-ISSUED: November 6, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
LaRosa; Gregory J.	West Roxbury	MA		

US-CL-CURRENT: 424/130.1; 424/141.1, 424/143.1, 424/159.1, 530/388.22, 530/388.23, 530/389.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 7. Document ID: US 5789539 A

L2: Entry 7 of 8 File: USPT Aug 4, 1998

US-PAT-NO: 5789539
DOCUMENT-IDENTIFIER: US 5789539 A

TITLE: Chemokine-like proteins and methods of use

DATE-ISSUED: August 4, 1998

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Daly; Thomas J.	Framingham	MA		
LaRosa; Gregory J.	Boston	MA		

US-CL-CURRENT: 530/324; 424/85.2, 435/69.52, 435/70.1, 435/71.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 8. Document ID: US 5656724 A

L2: Entry 8 of 8 File: USPT Aug 12, 1997

US-PAT-NO: 5656724
DOCUMENT-IDENTIFIER: US 5656724 A

TITLE: Chemokine-like proteins and methods of use

DATE-ISSUED: August 12, 1997

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Daly; Thomas J.	Framingham	MA		
LaRosa; Gregory J.	Boston	MA		

US-CL-CURRENT: 530/324; 424/85.2, 435/69.52

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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Terms	Documents
LaRosa Gregory J.in.	8

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Monoclonal
Anti-human CCR-2 Antibody

ORDERING INFORMATION

Catalog Number: MAB150

Clone: 48607.121

Lot Number: AOT02

Size: 500 µg

Formulation: 0.2 µm filtered solution in PBS

Storage: -20° C

Reconstitution: sterile PBS

Specificity: rhCCR-2

Immunogen: CCR-2 transfectants

Ig class: mouse IgG_{2B}

Applications: Flow Cytometry

Preparation
This antibody was produced from a mouse hybridoma elicited from a Balb/c mouse inoculated with hCCR-2 transfected NSO mouse myeloma cells. The IgG fraction of ascites fluid was purified by Protein G affinity chromatography.

Formulation
Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS).

Endotoxin Level
< 10 ng per 1 mg of the antibody as determined by the LAL method.

Reconstitution
Reconstitute with sterile PBS. If 1 mL of PBS is used, the antibody concentration will be 500 µg/mL.

Storage
Lyophilized samples are stable for greater than six months when held at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 4° C for at least 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C for at least six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity
This antibody has been selected for its ability to react specifically with hCCR-2 transfectants and not the parental cell line using FACS analysis. This antibody does not cross-react with CCR-5 transfectants.

Applications
Flow cytometry: This antibody can be used at 5 - 10 µg/mL and 10 µL/10^5 cells to detect human CCR-2.

Optimal dilutions should be determined by each laboratory for each application.

R&D Systems Inc.

1-800-343-7475

Backgr und Information

CCR-2 is a G-protein linked seven transmembrane domain spanning chemokine receptor that preferentially binds monocyte chemoattractant proteins-1 and 3 (MCP-1 and MCP-3) (1, 2). Two isoforms of this receptor (CCR-2A and CCR-2B) are expressed on cell surfaces as a result of alternate splicing from the same gene (1). These two CCR-2 variants differ only at their intracellular carboxyl terminals, with the CCR-2A form possessing 14 additional amino acids. This may provide a mechanism by which cells responding to similar extracellular ligands can activate different intracellular second messengers. Cells that respond to the action of MCP-1 and therefore are likely to express CCR-2 receptors, include monocytes, T cells, NK cells, basophils, mast cells and dendritic cells (3,4). A recent report suggests that B cells may also express CCR-2 receptors (5). The recognition that a variety of chemokine receptors, including CCR-2, can serve as HIV fusion co-factors (6) and as facilitators of T cell recruitment during inflammation (7) makes chemokine receptor monitoring an important exercise in elucidating the HIV infection process and the regulation of inflammatory reactions.

References

1. Charo, I.F. *et al.* (1994) Proc. Natl. Acad. Sci USA **91**:2752.
2. Myers, S.J. *et. al.* (1995) J. Biol. Chem. **270**:5786.
3. Jiang, Y. *et al.* (1992) J. Immunol. **148**:2423.
4. Locati, M. *et al.* (1994) J. Biol. Chem. **269**:4746.
5. Frade, J.M.R. *et al.* (1997) J. Immunol. **159**:5576.
6. Doranz, B.J. *et al.* (1996) Cell **85**:1149.
7. Loetscher, P. *et al.* (1996) J. Exp. Med. **184**:569.

Note: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.

Monoclonal anti-human CCR-2-Phycoerythrin

Catalog Numb r: FAB151P
100 tests

Reagent Information

Phycoerythrin-conjugated mouse monoclonal anti-human CCR-2: contains 1.0 mL of PE-labeled antibody, at a concentration of 50 µg/mL.

Clone #: 48607.211

Storage: 2-8 °C

Ig class: mouse IgG_{2B}

Additional Reagents Required

- PBS (Dulbecco's PBS)
- BSA

Intended Use

Designed to quantitatively determine the percentage of cells bearing the cell surface receptor CCR-2 within a population and qualitatively determine the density of this receptor on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the PE-labeled monoclonal antibody, which binds to the cells expressing the CCR-2 receptor. Unbound PE-conjugated antibody is then washed from the cells. Following the use of a secondary developing reagent like avidin-FITC or streptavidin-PE, cells expressing the CCR-2 structure are fluorescently stained, with the intensity of staining directly proportional to the density of the CCR-2. Cell surface expression of the CCR-2 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

PE-conjugated mouse anti-human CCR-2: Use as is; no preparation necessary.

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anti-coagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. 50 µL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Whole blood cells will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4×10^6 cells/mL and 25 µL of cells (1×10^5) are transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

1) Cells to be used for staining with the antibody may be first Fc-blocked by treatment with 1 µg of human IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction
2) Transfer 25 µL of the Fc-blocked cells (1×10^5 cells) or 50 µL of packed whole blood to a 5 mL tube.
3) Add 10 µL of biotin-conjugated anti-CCR-2 reagent.
4) Incubate for 30-45 minutes at 2 - 8° C.
5) Following this incubation, remove unreacted anti-CCR-2 reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Cat #: WL1000*).
6) Resuspend cells in approximately 100 µL of PBS buffer and then add 10 µL of either avidin-FITC (10 µg/mL stock) or streptavidin-PE (5 µg/mL stock) and allow the reaction to develop for 30 minutes at 2 - 8° C.
7) Finally, wash the cells as step 5 above and resuspend the cells in 200-400 µL of PBS buffer for final flow cytometric analysis.
8) As a control for analysis, cells in a separate tube should be treated with PE-labeled mouse IgG_{2B} antibody.

This procedure may need to be modified, depending upon final utilization.

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Background Information

CCR-2 is a G-protein linked seven transmembrane domain spanning chemokine receptor that preferentially binds monocyte chemoattractant proteins-1 and 3 (MCP-1 and MCP-3) (1, 2). Two isoforms of this receptor (CCR-2A and CCR-2B) are expressed on cell surfaces as a result of alternate splicing from the same gene (1). These two CCR-2 variants differ only at their intracellular carboxyl terminals, with the CCR-2A form possessing 14 additional amino acids. This may provide a mechanism by which cells responding to similar extracellular ligands can activate different intracellular second messengers. Cells that respond to the action of MCP-1 and therefore are likely to express CCR-2 receptors, include monocytes, T cells, NK cells, basophils, mast cells and dendritic cells (3,4). A recent report suggests that B cells may also express CCR-2 receptors (5). The recognition that a variety of chemokine receptors, including CCR-2, can serve as HIV fusion co-factors (6) and as facilitators of T cell recruitment during inflammation (7) makes chemokine receptor monitoring an important exercise in elucidating the HIV infection process and the regulation of inflammatory reactions.

References

1. Charo, I.F. *et al.* (1994) Proc. Natl. Acad. Sci USA **91**:2752.
2. Myers, S.J. *et. al.* (1995) J. Biol. Chem. **270**:5786.
3. Jiang, Y. *et al.* (1992) J. Immunol. **148**:2423.
4. Locati, M. *et al.* (1994) J. Biol. Chem. **269**:4746.
5. Frade, J.M.R. *et al.* (1997) J. Immunol. **159**:5576.
6. Doranz, B.J. *et al.* (1996) Cell **85**:1149.
7. Loetscher, P. *et al.* (1996) J. Exp. Med. **184**:569.

Note: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.

Monoclonal
Anti-human CCR-2 Antibody

ORDERING INFORMATION

Catalog Number: MAB150

Clone: 48607.121

Lot Number: AOT02

Size: 500 µg

Formulation: 0.2 µm filtered solution in PBS

Storage: -20° C

Reconstitution: sterile PBS

Specificity: rhCCR-2

Immunogen: CCR-2 transfectants

Ig class: mouse IgG_{2B}

Applications: Flow Cytometry

Preparation
This antibody was produced from a mouse hybridoma elicited from a Balb/c mouse inoculated with hCCR-2 transfected NSO mouse myeloma cells. The IgG fraction of ascites fluid was purified by Protein G affinity chromatography.

Formulation
Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS).

Endotoxin Level
< 10 ng per 1 mg of the antibody as determined by the LAL method.

Reconstitution
Reconstitute with sterile PBS. If 1 mL of PBS is used, the antibody concentration will be 500 µg/mL.

Storage
Lyophilized samples are stable for greater than six months when held at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 4° C for at least 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C for at least six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity
This antibody has been selected for its ability to react specifically with hCCR-2 transfectants and not the parental cell line using FACS analysis. This antibody does not cross-react with CCR-5 transfectants.

Applications
Flow cytometry: This antibody can be used at 5 - 10 µg/mL and 10 µL/10^5 cells to detect human CCR-2.

Optimal dilutions should be determined by each laboratory for each application.

R&D Systems Inc.

1-800-343-7475

Monoclonal anti-human CCR-2-Biotin

Catalog Numb r: FAB151B
100 t sts

Reagent Information

Biotin-conjugated mouse monoclonal anti-human CCR-2: contains 1.0 mL of biotin-labeled antibody, at a concentration of 50 µg/mL.

Clone #: 48607.211

Storage: 2-8 °C

Ig class: mouse IgG_{2B}

Additional Reagents Required

- PBS (Dulbecco's PBS)
- BSA

Intended Use

Designed to quantitatively determine the percentage of cells bearing the cell surface receptor CCR-2 within a population and qualitatively determine the density of this receptor on cell surfaces by flow cytometry.

Principle of the Test

Washed cells are incubated with the biotin-labeled monoclonal antibody, which binds to the cells expressing the CCR-2 receptor. Unbound biotin-conjugated antibody is then washed from the cells. Following the use of a secondary developing reagent like avidin-FITC or streptavidin-PE, cells expressing the CCR-2 structure are fluorescently stained, with the intensity of staining directly proportional to the density of the CCR-2. Cell surface expression of the CCR-2 is determined by flow cytometric analysis using 488 nm wavelength laser excitation.

Reagent Preparation

Biotin-conjugated mouse anti-human CCR-2: Use as is; no preparation necessary.

Sample Preparation

Peripheral blood cells: Whole blood should be collected in evacuated tubes containing EDTA or heparin as the anti-coagulant. Contaminating serum components should be removed by washing the cells three times in an isotonic phosphate buffer (supplemented with 0.5% BSA) by centrifugation at 500 x g for 5 minutes. 50 µL of packed cells are then transferred to a 5 mL tube for staining with the monoclonal. Whole blood cells will require lysis of RBC following the staining procedure.

Cell Cultures: Continuous cell lines or activated cell cultures should be centrifuged at 500 x g for 5 minutes and washed three times in an isotonic PBS buffer (supplemented with 0.5% BSA), as described above, to remove any residual growth factors that may be present in the culture medium. Cells should then be resuspended in the same buffer to a final concentration of 4×10^6 cells/mL and 25 µL of cells (1×10^5) are transferred to a 5 mL tube for staining.

Note: Adherent cell lines may require pretreatment with 0.5 mM EDTA to facilitate removal from substrate. Cells that require trypsinization to enable removal from substrate should be further incubated in medium for 6 - 10 hours on a rocker platform to enable regeneration of the receptors. The use of the rocker platform will prevent reattachment to the substrate.

Sample Staining

1) Cells to be used for staining with the antibody may be first Fc-blocked by treatment with 1 µg of human IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction
2) Transfer 25 µL of the Fc-blocked cells (1×10^5 cells) or 50 µL of packed whole blood to a 5 mL tube.
3) Add 10 µL of biotin-conjugated anti-CCR-2 reagent.
4) Incubate for 30-45 minutes at 2 - 8° C.
5) Following this incubation, remove unreacted anti-CCR-2 reagent by washing (described above) the cells twice in 4 mL of the same PBS buffer (*note that whole blood will require a RBC lysis step at this point using any commercially available lysing reagent, such as R&D Systems Whole Blood Lysing Kit, Cat #: WL1000*).
6) Resuspend cells in approximately 100 µL of PBS buffer and then add 10 µL of either avidin-FITC (10 µg/mL stock) or streptavidin-PE (5 µg/mL stock) and allow the reaction to develop for 30 minutes at 2 - 8° C.
7) Finally, wash the cells as step 5 above and resuspend the cells in 200-400 µL of PBS buffer for final flow cytometric analysis.
8) As a control for analysis, cells in a separate tube should be treated with biotin-labeled mouse IgG_{2B} antibody.

This procedure may need to be modified, depending upon final utilization.

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1-800-343-7475